J. Vet. Anim. Sci. (2012), Vol. 2: 72-78

Post-Vaccinal Observation of Lymphoid Organs in Broiler Chicks Inoculated with Hot and Mild Vaccinal Strains of Infectious Bursal Disease Virus

S. Azhar*, S. Akhtar and M. A. Munir

*Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

ABSTRACT

This project was carried out to study the effect of two intermediate plus (hot) vaccines (228-E and BUR-706) and one mild (Gumboral CT) vaccinal strain of Infectious Bursal Disease virus (IBDV) on antibody response and their effect on different lymphoid organs including bursa, spleen and thymus in chickens. Chicks (n=250) were divided into 4 groups and vaccinated with these strains of IBDV on day 15th of their age and antibody levels were monitored using indirect haemagglutination (IHA) test every week up to 5th weeks of their age. On days 25th post vaccination, IHA results revealed that the birds vaccinated with 228-E and Bur-706 had significantly higher antibody titers, respectively as compared to those vaccinated with Gumboral CT. Reduction in total body weights and lymphoid organs-body weight ratios were recorded, showing that intermediate strains were more damaging than milder one. Histopathological studies also showed severe damage in bursae of broilers inoculated with intermediate plus vaccines than milder. On 25th day of age, five birds from each treatment group were challenged with a virulent IBDV field isolate. After seven day post-challenge, high morbidity and mortality was observed in control group than vaccinated groups. This study suggested the use of intermediate strains as vaccine since they induced high antibody titers as compared to that of the milder strain. However, more invasive and pathogenic intermediate strains showed adverse effects on the development of lymphoid organs harboring B cells. In conclusion, there is a need to develop an effective infectious bursal disease vaccine, low in virulence, which could be used for mass vaccination in chickens, conferring excellent protection against the disease with minimum immunosuppressive effects.

***Key Words*:** Infectious bursal disease; Intermediate plus vaccines; histopathological examination; protective efficacy; immunosuppression

INTRODUCTION

Infectious bursal disease is a viral infection, affecting the immune system of poultry. The disease is highly contagious, affects young chickens, and is characterized by the destruction of the lymphoid organs, particularly the bursa of Fabricius, where B lymphocytes mature and differentiate (van den Berg et al., 2000). The infectious bursal disease virus (IBDV) is the etiological agent of "Gumboro disease" (Muller et al., 2003). The IBDV is a two segmented double-stranded RNA virus and belongs to family *Birnaviridae* (Carballeda et al., 2011). There are two recognized serotypes of IBDV, serotype 1 and II. Only serotype 1 has been known to cause naturally occurring disease in chicken (Wyeth and Chettle, 1988). Post mortem changes include marked hemorrhages at proventriculo-gizzard junction and swelling of bursa which may be twice than normal in size, possibility due to edema and hyperemia (Cheville, 1967). The IBD intermediate type vaccines are considered to be more invasive, replicative and immunogenic than milder one (Giambrone and Clay, 1985). However,

*Corresponding author
shajeelazhar@hotmail.com

these vaccines have adverse effect on lymphoid organs. Reduction of bursal weight of vaccinated groups is associated with the proliferation of the virus vaccine in the bursal tissues. Intermediate-plus vaccine cause severe injury to bursa of Fabricius in vaccinated birds (Abu-Tabeekh and Al-Mayah, 2009). Keeping in view, this study was focused on the evaluation of different strains including two intermediate plus (hot) and one mild strain of Infectious Bursal Disease virus, in an effort to rule out the better one, for vaccine production with higher protective efficacy in terms of antibody response and development of lymphoid organs.

MATERIALS AND METHODS

Experimental Birds

A total of 250 day-old broiler chicks were reared at the Experimental House, University of Veterinary and Animal Sciences, Lahore, Pakistan and provided feed and water ad libitum.

Vaccines and Vaccination

The experimental birds were randomly divided into four groups i.e. A, B, C and D. Moreover, all the groups were also vaccinated against Newcastle disease and hydropericardium syndrome according to the routine schedule.

On day 15, the birds of Groups A, B and C were vaccinated with IBD vaccine of 228-E strain, Bur 706 strain and Gumboral CT strain respectively. The birds of Group D were served as control

Post Vaccinal Observations

Indirect Haemagglutination Test

Ten blood samples were collected from each group, at an interval of 4 days starting from day one till 35 days. The antibody titres against respective IBDV strains were detected by indirect haemagglutination (IHA) test by following the procedure described by Gold and Fudenberg (1967).

Histopathological Studies

Five birds from each group were randomly isolated with an interval of four days starting from day one to 35th day and killed to observe the histopathological changes in lymphoid organs including bursa, spleen and thymus (Drury and Willington, 1980).

Table 1 Determination of Embryo Infective Dose EID_{50}

Groups	Dilution	Embryonated eggs	Died	Survivor	Accumulated Number			
					Died	Survivor	Mortality Ratio	Mortality (%)
A	10^{-1}	5	5	0	24	0	24/24	100.0
B	10^{-2}	5	5	0	19	0	19/19	100.0
C	10^{-3}	5	5	0	14	0	14/14	100.0
D	10^{-4}	5	3	2	9	2	9/11	81.8
E	10^{-5}	5	2	3	6	5	6/11	54.5
F	10^{-6}	5	2	3	4	8	4/12	33.3
G	10^{-7}	5	1	4	2	12	2/14	14.3
H	10^{-8}	5	1	4	1	16	1/17	5.9

$EID_{50}= 10^{-5.214}$ /ml

Lymphoid Organs-body Weight Ratios

A comparison of lymphoid organs-body weight ratios of birds in all groups was made at day 20th post vaccination. The IBDV antigen was obtained from field outbreaks of the disease and used for calculation of

Embryo infective dose (EID_{50}) according to Reed and Muench (1938).

Challenge Experiment: On day 25th post vaccination, five birds from each group were separated randomly and were challenged with field isolate of IBDV at a dose of EID_{50} i.e. $10^{-5.214}$ and kept under observation for seven days. The challenged live and dead birds were killed and postmortem examination was performed to record lesion scores in lymphoid organs.

Statistical Analysis

The data collected was analyzed statistically by using ANOVA (Steel and Torrie, 1982).

RESULTS

Antibody Titres

Results showed the highest antibody titer on day 35 of age in groups inoculated with intermediate plus vaccines than mild vaccinal strain

Lymphoidal Organs-body weight Ratios

Mean lymphoid organs-body weight ratios of birds in all groups showed significantly lower bursa-body weight ratio at day 20th post vaccinations but no significant difference was observed for mean thymus- and spleen-body weight ratios.

Histopathological Findings

In all the groups, no histopathological changes were observed on bursa before vaccination; whereas, certain pathological changes were observed post vaccination. Spleen and thymus appeared normal in pre-vaccination period; but slight changes were observed in spleen tissue post-vaccination.

Post-challenge Findings

The birds from all groups were challenged on day 25 to determine the protective efficacy of vaccines. After seven day post-challenge a comparison was made between challenge morbidity and mortality in various treatment groups. The gross appearance of bursa in the challenged non-vaccinated birds revealed increase in size due to edema and hemorrhagic striations. Histopathological findings in all groups showed severe damage in bursal tissues; however, damages recovered in all the groups rapidly except in the control group. Spleen and thymus were slightly swollen grossly in all groups but in the control group, small grey necrotic foci dispersed on its entire surface were also observed.

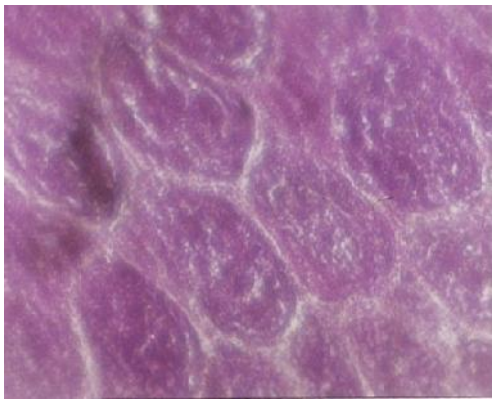
Table 2 IBD, IHA antibody titers in chickens of various treatment groups at different age interval

Group	Days indicating GMT IHA							
	1	5	10	15	20	25	30	35
A	5.3^{a}	1.41^{a}	1.14^{a}	0	1.14^{a}	4.0^{a}	6.5^{a}	8.0^{a}
B	2.0^{a}	2.0^{a}	1.51^{a}	0	1.14^{a}	3.5^{a}	5.7^{a}	7.5^{a}
C	5.3^{a}	1.62^{a}	1.51^{a}	0	1.07^{a}	1.74^{b}	2.5^{b}	3.0^{b}
D	3.5^{a}	2.14^{a}	1.31^{a}	0	0	0	1.07^{c}	1.14^{c}

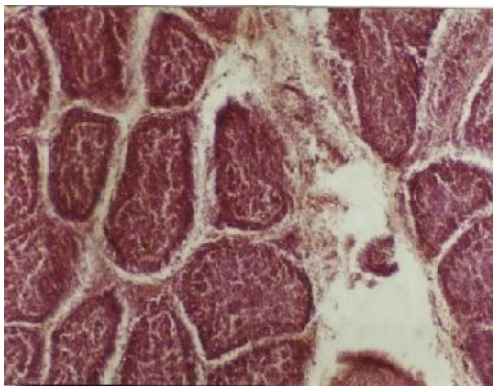
Values with same superscript are not significantly different ($p < 0.05$) GMT: Geometric mean titer

Table 3 Comparison of mean bursal, splenic and thymic body weight ratios on day 35

Group	Bursal	Splenic	Thymic
A	0.990 ± 0.047^{b}	1.41 ± 0.212^{b}	1.62 ± 0.055^{b}
B	1.046 ± 0.049^{b}	1.48 ± 0.266^{ab}	1.71 ± 0.133^{b}
C	1.072 ± 0.068^{b}	1.65 ± 0.080^{ab}	1.99 ± 0.338^{ab}
D	2.554 ± 0.207^{a}	2.99 ± 0.155^{a}	2.43 ± 0.229^{a}

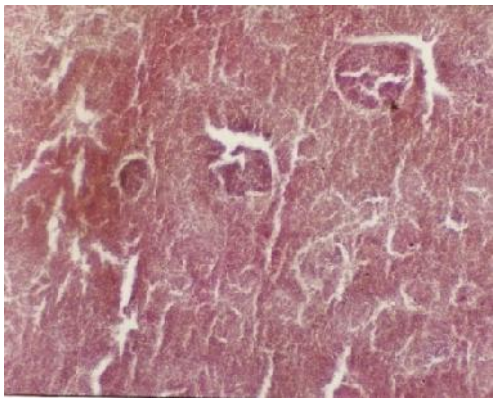


1a. Healthy chicken



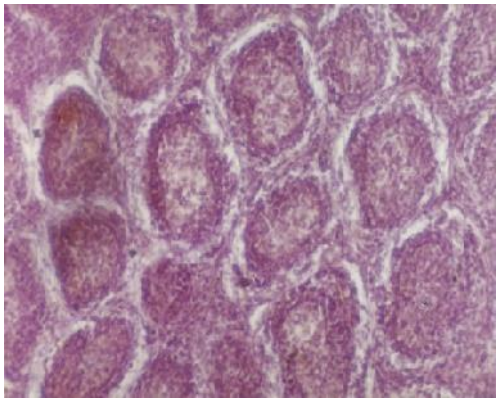
1b. IBD affected chicken

Figures 1 (a-b) Bursa of fabricius of healthy and IBD affected chickens (H&E staining)

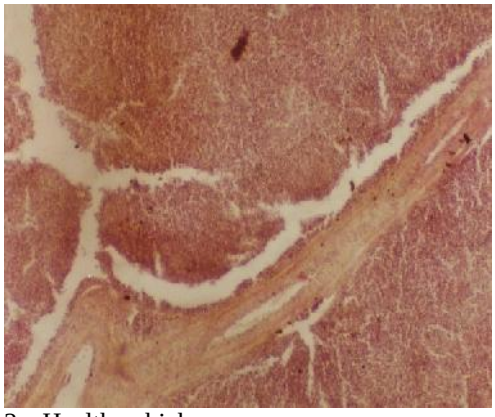


2b. IBD affected chicken

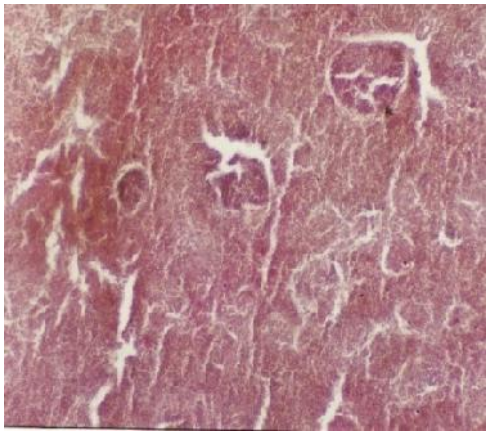
Figures 2 (a-b): Spleen of healthy and IBD affected chickens (H&E staining)



3a. Healthy chicken



2a. Healthy chicken



3b. IBD affected chicken

Figures 3 (a-b) Thymus of healthy and IBD affected chickens (H&E staining)

Table 4 Histopathological lesions on bursa among various treatment groups

Groups	Days																				
	20					25					30					35					
	LD	MP	OIFS	EPIT	CN	LD	MP	OIFS	EPIT	CN	LD	MP	OIFS	EPIT	CN	LD	MP	OIFS	EPIT	CN
A	+	+	±	+	+	+	+	+	+	+	±	+	+	±	.	.	±	+	+	.
B	+	+	+	.	.	+	+	±	+	.	+	+	+	+	+	.	+	+	+	.
C	.	.	.	.	.	+	+	±	.	.	+	+	+	±	.	+	+	+	+	+
D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

LD=Lymphoid Depletion, EPIT=Epithelium Thickness, OIFS=edematous Interfollicular space, CN=Coagulative Necrosis, MP=Macrophages Presence

Table 5 Morbidity and mortality percentage of birds on day 7th post-challenge with IBDV

Groups	Morbidity (%)	Mortality (%)
A	60%	40%
B	60%	40%
C	60%	40%
D	20%	80%

DISCUSSION

Infectious bursal disease is a viral infection of chickens causing degeneration of bursa of Fabricius that results in suppression of humoral immune responses. The most effective vaccines for chickens, with maternal antibodies are live vaccines. The live virus replication stimulates the immunogenic response more than killed viruses and antibody titers are higher. Intermediate strain vaccines (228-E and BUR-706) performed better as this vaccine induced significantly higher antibody levels than vaccine with mild strain and similar findings have been reported by Al-Zubeedy (2009). Although, all vaccines in this study contained live virus strains, but 228-E and BUR-706, being intermediate type, are considered to be more invasive, replicative and immunogenic than Gumboral CT. Consequently, these provided strong antigenic stimulus to the birds resulting in corresponding higher antibody titers. These findings are in line with that of Giambrone and Clay (1985) and Gregorio (1994) who reported similar results. On the other hand, vaccines containing more virulent (hot) strains usually end up in irreversible damages. Histopathological studies of lymphoid organ indicated that the vaccines induced bursal damage after vaccination. These vaccines induced the bursal atrophy is also indicated by other studies (Samanta et al., 2011).

In challenge experiment, the birds from control non-vaccinated group showed 80% mortality. Grossly bursa of the control group showed lesions but histopathological damages appeared in all the groups which recovered rapidly in vaccinated groups but did not recover in non-vaccinated chickens. Grossly, the spleen and thymus of vaccinated groups appeared normal but slight lesions were observed in non vaccinated groups on microscopic examination. These results are in line with study of Tartar et al. (1995) who showed that antibody titers of vaccinated groups were protective than the unvaccinated ones but severe lesions were observed on bursa of Fabricius of vaccinated chickens which revealed the immunosuppressive effect of intermediate plus vaccine. These findings are in agreement with the findings of Boudaoud et al. (2008).

Based upon the findings of the present study, it may be concluded that intermediate plus strains of IBDV have good protective efficacy against infectious bursal disease but cause bursal atrophy. So, the new vaccines like rationally designed subunit and/or recombinant viral vector vaccines may be a good replacement to avoid such adverse effects induced by live vaccines.

REFRENCES

Al-Zubeedy, Z. 2009. Immune response in day-old broiler chicks vaccinated

against Newcastle disease virus. *Iraqi Journal of Veterinary Sciences*, 23: 143-146.

Boudaoud, A., and N. Alloui. 2008. Evaluation of the safety of live attenuated vaccine viruses against infectious bursal disease (Gumboro disease) in conventional broiler chicks. Département vétérinaire, Faculté des sciences, Université El-Hadj Lakhdar, Batna 5000, Algérie.

Carballeda, J. M., S. C. Zoth, E. Gomez, M. J. Gravisaco, and A. Berinstein. 2011. Activation of the immune response against Infectious Bursal Disease virus after intramuscular inoculation of an intermediate strain. Instituto de Biotecnología, Buenos Aires, Argentina.

Cheville, N.F. 1967. Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of the chicken. *American Journal of Pathology*, 51: 527-551.

Drury, R. A. B. and E. A. Wellington. 1980. Carleton's Histological Technique, 5th edn, Oxford University Press

Giambrone, J. J. and R. P. Clay. 1985. Evaluation of the immunogenicity, stability, pathogenicity and immunodepressive potential of four commercial live IBDV. *Poultry Science*, 65: 1287-1290.

Gold, E. R. and H. H. Fudenberg. 1967. Chromic chloride: A coupling reagent for passive hemagglutination reaction. *Journal of Immunology*, 99: 859-866.

Gregorio, R. 1994. Control program and assessment of immunity in broilers and broiler breeders. *World Poultry Magazine*, pp: 21-23.

Abu-Tabeekh, M. A. S. and A. A. S. Al-Mayah. 2009. Morphological investigation of bursa of Fabricius of imported broilers and local chicks vaccinated with two types of IBD vaccines. *Iraqi Journal of Veterinary Sciences*, 23: 201-206.

Muller, H., Islam, M. R. and Raue, R. 2003. Research on infectious bursal disease-the past, the present and the future. Institute for Virology, Faculty of Veterinary Medicine, University of Leipzig, Germany.

Reed, L. J. and H. Muench. 1938. A simple method of estimating fifty percent and point. *American Journal of Hygiene*, 27: 493-496.

Samanta, A., D. Niyogi, S. Ganguly, Y. D. Singh, and S. Pal. 2011. Study on Immunosuppressive effect of vaccination against infectious bursal disease. *Indian Journal of Veterinary Pathology*, 35: 227-229.

Steel, R. G. D. and J. H. Torrie. 1982. In: Principles and Procedures of Statistics, A biochemical approach. 2nd. Ed., McGraw Hill International. Book Co., USA.

Tatar, N. D. and S. A. Khan. 1995. How to control infectious bursal disease in poultry. *Proceedings of National Symposium of Veterinary Pathologists* (April 6th, 1995), Lahore, Pakistan, pp: 48-49.

Vanden B., T. P., N. Eterradossi, D. Toquin, and G. Meulemans. 2000. Infectious bursal disease (Gumboro disease) (1) Section de Virologie Aviaire, Centre d' Études et de Recherches Vétérinaires et Agrochimiques, Groeselenberg, 1180 Brussels, Belgium, Germany.

Wyeth, P. J. and N. J. Chettle. 1988. Ager gel diffusion test for differentiation between straina of type-1 IBD virus. *Veterinary Record*, 122: 442-443.